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CD34 AND C-KIT IMMUNOREACTIVE CELLS IN THE HUMAN EMBRYONAL AND FETAL SMALL BOWEL

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Abstract. *Interstitial cells of Cajal (ICC) play important roles in the control of digestive motility: they generate the electrical slow-wave activity (pacemaker component) of the gut musculature and are involved in neurotransmission and stretch sensation. ICC expresses c-kit and depends on signaling via Kit receptors for development and maintenance of phenotype. The aim of the present study was to investigate if the c-kit immunoreactive (IR) cells present in the wall of the small bowel at the beginning of the fetal period are CD34 immunopositive. Human small bowel specimens were obtained from 5 embryos and 7 foetuses, 7–12 weeks of gestational age. The specimens were exposed to anti-c-kit antibodies to investigate ICC differentiation and anti-CD34 antibodies to identify presumed ICC progenitors. The differentiation of smooth muscle cells was studied with anti-desmin antibodies. At 9–10 weeks, c-kit IR cells were present in the wall of small bowel in the form of a narrow band of cells, at the level of the myenteric plexus, but they were absent in the mucosa and submucosa of the gut. At the same time, CD34 IR cells were present at the level of submucosa and mucosa, and they were not present in the outer parts of gut wall. A clear distinction between the localization of c-kit IR cells and CD34 IR cells was evident. We may conclude that c-kit IR cells present in the small bowel wall at the beginning of fetal period of development, at 9–10 weeks, do not exhibit concurrent CD34 immunoreactivity.*

Key words: *Small bowel, c-kit, CD-34, immunohistohemistry, human*

Introduction

Interstitial cells of Cajal (ICC) are a distinct and unique cell population distributed in the muscle layer of digestive tube of many vertebrates including humans [1, 2]. They are network-forming cells connected electrically with each other and with smooth muscle cells via gap junctions [3, 4]. ICC play important roles in the control of digestive motility: they generate the electrical slow-wave activity (pacemaker component) of the gut musculature [5–7] and are involved in neurotransmission [8, 9] and stretch sensation [10]. ICC express c-kit and depend on signaling via Kit receptors for development and maintenance of phenotype [11].

At the end of the embryonic period of human development, c-kit immunoreactive (c-kit IR) cells are present in the oesophagus and stomach wall in the form of a wide belt of cells around the inception of the myenteric plexus (MP) ganglia [12, 13]. In the small and large bowel, c-kit-IR cells appear later (in the small bowel at 9 weeks, and in the colon at 10–12 weeks), in the form of narrow linear rows of cells, also in the MP region [14–16].

Enteric neurons and glial cells arise from the neural crest (from "vagal" and "sacral" level) [17]. Lecoin et al. [18] were the first to show that ICC in the avian intestine do not arise from the neural crest. The study by Klüppel et al. [19] also suggested strongly that ICC and smooth muscle cells arise from common precursor cells. Smooth muscle markers, such as the heavy chain of smooth muscle myosin, are coexpressed with Kit in the developing gut [19]. However, some of the Kit-positive mesenchymal cells are destined to become smooth muscle cells, and such cells down-regulate the expression of Kit and unregulate the expression of myofilament proteins. ICC maintains their expression of Kit, even in mature animals.

Lorincz et al. [20] provided evidence for characterization of potential progenitor cells for ICC in the stomach of adult mice. They have demonstrated that these cells are positive for Kit, CD34 (an adhesion molecule), as well as CD44, insulin receptors and IGF-I receptors (IGF-IR) [20]. CD34-cells, mostly known as interstitial Cajal-like cells (ICLCs), are present in the submucosa of the entire human gastrointestinal tract [21]. Recently, telocytes, belonging to the group of ICLCs, were described as a distinctive type of cells [22]. The ICC precursor cell could possibly appear as a fibroblast-like (immature)

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ICC at the ultrastructural level, identified in W mutant animals; these cells are Kit negative, but have gap junction contact with smooth muscle cells and are also associated with Auerbach's plexus [23].

The identification of the morphology of the immature ICC, their cytological changes and their organization during differentiation might help in interpreting the significance of abnormalities in the ICC distribution, density and morphology at birth and in the early paediatric age, as well as in understanding the pathophysiology of intestinal motile disorders in neonates and young children.

The aim of the present study was to investigate if the c-kit IR cells present in the wall of the small bowel at the beginning of the foetal period are CD34 immunopositive, respectively if they represent the common precursors of ICC and smooth muscle cells, or already differentiated, mature ICC.

Material and Methods

The human material was obtained after legal abortions (0.5–1 h postmortem) and premature births due to pre-partial deaths according to the principles of the Ethical Committee of the Faculty of Medicine of the University of Niš. Both genders are represented in the sample, and no specimens had gastrointestinal disorders. Gestational ages were estimated by anatomic criteria according to the Carnegie Staging system and the crown-rump length, head circumference, and foot length. Each embryo and foetal small bowel specimen was fixed in 10% neutral formalin and paraffin-embedded. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Niš.

The study material consisted of 5 human embryos and 7 human foetuses, 7–12 weeks gestational age (7 weeks, n=2; 8 weeks, n=3; 9 weeks, n=2; 10 weeks, n=2; 12 weeks, n=3). Small foetuses (9 and 10 weeks) were processed completely, sequentially sectioned at 4 μm, and stained. Immunohistochemical analysis was performed using the detection Kit-Polymer. The sections were deparaffined in xylol and a descending series of alcohol rinses (< 1 min each), then rehydrated in distilled water. The endogenous peroxidase was blocked with 3% H_2O_2 for 10 min at room temperature. This was followed by incubation with the primary antibodies for 60 min at room temperature, rinsing in a phosphate buffered solution (0.1M PBS, pH 7.4). The primary antibodies were dissolved in Dako antibody diluent (Cat. No. S0809). The sections were incubated with streptavidin horseradish conjugate for 30 min at room temperature. The complex was visualised with DAKO Liquid DAB + Substrate/ Chromogen System (Code No. K3468) and DAKO AEC + Substrate/Chromogen System (code no. K3469; Dako). Immunostaining for CD34 was then performed as previously described. All immunolabelled sections were counterstained by Mayer's haematoxylin. Immunoreactivity was absent in negative controls in which the primary antibody was omitted. Sections were examined with an Olympus BX50 microscope and photographed with an Olympus PM-C35 camera.

The primary antibodies used, and their respective dilutions, are listed in Table 1.

Table 1. Antibodies

Antigen	Clone	Supplier	Dilution
C-kit	CD-117	Dako	1 : 300
CD34	QBEnd 10 N1632	Dako	Ready to use
Desmin	DE-R-11	Dako	1 : 100

Results

In the study, we have not considered the initial portions of the small bowel, immediately adjacent to the stomach, developing from the foregut, in which ICC differentiate in the way identical to that in the stomach.

At the end of the embryonic period of development, at weeks 7 and 8, DES immunoreactivity was faint in the cells that would form the circular muscle layer. In the same period, c-kit IR cells were absent in the wall of the midgut, part of the primitive gut which gives a rise to small bowel. CD34 IR cells were present in the inner parts of the wall of the primitive gut, which will develop in mucosa and submucosa, but not present at the level of the MP ganglia. At 9–10 weeks, DES immunoreactivity was present in all parts of the small bowel. DES immunostaining was observed as a band that encircled the gut (corresponded to the circular muscle layer), and in the form of an extremely thin band of cells located outside the MP (presumptive longitudinal muscle layer) (Fig. 1).

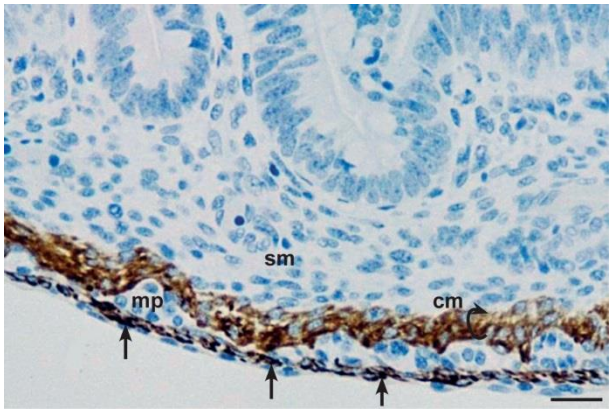

Fig. 1. Desmin immunohistochemistry (9 weeks gestational age, small bowel). DES-IR was present in the circular muscle layer and in the very thin band of cells located outside the MP, presumptive longitudinal muscle layer (*arrows*). cm, circular muscle layer; mp, myenteric plexus; sm, submucosa. Bar: 30 μm.

In this period of development, c-kit IR cells were detected in the wall of small bowel. They were present in the form of a narrow band of cells, at the level of the MP and encircled the ganglia, but neither their bodies nor their processes were present within the ganglia. C-kit IR cells were absent in the mucosa and submucosa of the gut (Fig. 2).

At the same time, CD34 IR cells were present and distributed in the identical way as in the embryonal period, at the level of submucosa and mucosa, and they were not present in the outer parts of gut wall (Fig. 3). A clear distinction between the localization of c-kit IR cells and CD34 IR cells was evident (Figs. 2 and 3).

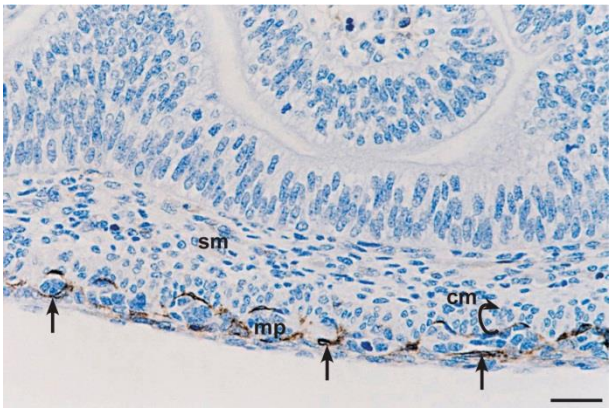

Fig. 2. C-kit immunohistochemistry **(**9 weeks gestational age, small bowel). C-kit-IR cells (*arrows*) located in the outer layers of the developing small bowel, surrounding the presumptive myenteric ganglia. cm, circular muscle layer; mp, myenteric plexus; sm, submucosa. Bar: 30 µm.

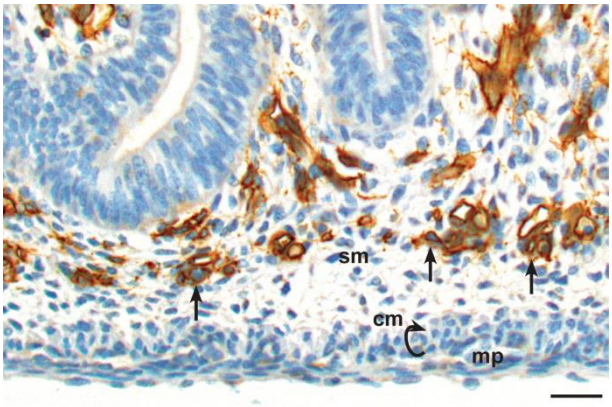

Fig. 3. CD34 immunohistochemistry (9 weeks gestational age, small bowel). CD34 IR cells (*arrows*) were present in the inner parts of the wall of the primitive gut, which will develop in mucosa and submucosa, but not present at the level of the MP ganglia. cm, circular muscle layer; mp, myenteric plexus; sm, submucosa. Bar: 30 µm.

By weeks 11–12, differences in the distribution of c-kit IR cells and CD34 IR cells which were described above, were present along the entire length of the small bowel.

Discussion

The paper described the time and mode of differentiation of muscle layers of the small bowel wall, primarily aiming to determine in a more precise way the place of appearance and mode of distribution of c-kit IR cells. Namely, at the end of embryonic period, the circular muscle layer differentiated, while the longitudinal appeared at the beginning of foetal period of development. These findings are in agreement with previous results regarding the development of neuromuscular structures in the human small bowel [24, 25].

Our results demonstrated that c-kit IR cells appeared in the small bowel wall (with the exception of its initial portion due to reasons mentioned above) not till the foetal period of development and in the myenteric plexus region, without being present in the inner parts of the wall, in the region of submucosa and mucosa of the bowel. On the other hand, CD34 IR cells were present at the end of embryonic period and the beginning of foetal period, but in both cases only in the inner parts of the small bowel wall, while they were not demonstrated in its outer parts. A difference in distribution of c-kit IR and CD34 IR cells in the small bowel wall was evident—c-kit IR cells did not exhibit simultaneous CD34 immunoreactivity. If the assumption of Huizinga and White [26] was applicable to the ICC development in humans, our findings would indicate that c-kit IR cells described in the small bowel wall in weeks 9–10 are in fact already differentiated, mature ICC. In other words, ICC precursors exhibit simultaneous c-kit and CD34 immunoreactivity, while mature ICC forms cease to exhibit CD34 and retain only c-kit IR properties [26]. On the other hand, according to the assumption by Horiguchi and Komuro [23], ICC precursors are c-kit negative and they could possibly appear as fibroblast-like cells. In that case, ICC precursors could be present in the small bowel wall in embryonal period of development, at 7–8 weeks, and the process of differentiation occurs at the beginning of foetal period when c-kit positive cells appear representing mature ICC. At the same time, the appearance of c-kit IR cells and longitudinal muscle layer is in accordance with the hypothesis by Klüppel et al. [19] who have claimed that ICC and smooth muscle arise from common precursor cells. Common precursors of the above cells are present in the outer parts of the bowel wall at the end of embryonic period of development, and later, at the beginning of fetal period of development a portion of these cells differentiates into smooth muscle cells of the longitudinal layer, while the rest differentiate into ICC. Certainly, the hypotheses stated above require further confirmation.

Conclusion

We may say that c-kit IR cells present in the small bowel wall at the beginning of fetal period of development, at 9–10 weeks, do not exhibit concurrent CD34 immunoreactivity.

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References

1. Komuro T. Structure and organization of interstitial cells of Cajal in the gastrointestinal tract. J Physiol 2006; 576:653–658.
2. Radenković G, Nikolić I, Todorović V. Interstitial cells of Cajal - pacemakers of the intestinal musculature. Facta Universitatis: Series Medicine and Biology 2005; 12:1–5.
3. Beckett EAH, Takeda Y, Yanase H, Sanders KM, Ward SM. Synaptic specializations exist between enteric motor nerves and interstitial cells of Cajal in the murine stomach. J Comp Neurol 2005; 493:193–206.
4. Thuneberg L, Peters S. Toward a concept of stretch-coupling in smooth muscle. I. Anatomy of intestinal segmentation and sleeve contraction. Anat Rec 2001; 262:110–124.
5. Thuneberg L. Interstitial cells of Cajal: intestinal pacemaker cells? Adv Anat Embryol Cell Biol 1982; 71:1–130.
6. Maeda H, Yamagata A, Nishikawa S. Requirement of c-kit for development of intestinal pacemaker system. Development 1992; 116:369–375.
7. Huizinga JD, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 1995; 373:347–349.
8. Ward SM. Interstitial cells of Cajal in enteric neurotransmission. Gut 2000; 47: 40–43.
9. Iino S, Horiguchi K. Interstitial cells of Cajal are involved in neurotransmission in the gastrointesinal tract. Acta Histochem Cytochem 2006; 39:145–153.
10. Thuneberg L, Peters S. Toward a concept of stretch-coupling in smooth muscle. I. Anatomy of intestinal segmentation and sleeve contraction. Anat Rec 2001; 262:110–124.
11. Maeda H, Yamagata A, Nishikawa S. Requirement of c-kit for development of intestinal pacemaker system. Development 1992; 116:369–375.
12. Radenkovic G, Savic V, Mitic D, Grahovac S, Bjelakovic M, Krstic M. Development of c-kit immunopositive interstitial cells of Cajal in the human stomach. J Cell Mol Med 2010a; 14:1125–1134.
13. Radenkovic G, Ilic I, Zivanovic D, Vlajkovic S, Petrovic V, Mitrovic O. C-kit-immunopositive interstitial cells of Cajal in human embryonal and fetal oesophagus. Cell Tissue Res 2010b; 340:427–436.
14. Radenkovic G. Two patterns of development of interstitial cells of Cajal in the human duodenum. J Cell Mol Med. 2012; 16:185-192.
15. Radenkovic G, Abramovic M. Differentiation of interstitial cells of Cajal in the human distal colon. Cells Tissues Organs 2012; 196:463–469.
16. Abramovic M, Radenkovic G, Velickov A. Appearance of interstitial cells of Cajal in the human midgut. Cell Tissue Res 2014; 356:9–14.
17. Burns AJ, LeDouarin NM. Enteric nervous system development: analysis of the selective developmental potentialities of vagal and sacral neural crest cells using quail-chick chimeras. Anat Rec 2001; 262:16–28.
18. Lecoin L, Gabella G, Le Douarin N. Origin of the c-kit-positive interstitial cells in the avian bowel. Development 1996; 122:725–733.
19. Klüppel M, Huizinga JD, Malysz J, Bernstein A. Developmental origin and Kit-dependent development of the interstitial cells of Cajal in the mammalian small intestine. Dev Dyn 1998; 211:60–71.
20. Lorincz A, Redelman D, Horvath VJ, Bardsley MR, Chen H, Ordög T. Progenitors of interstitial cells of Cajal in the postnatal murine stomach. Gastroenterology 2008; 134:1083–1093.
21. Pieri L, Vannucchi MG, Faussone-Pellegrini MS. Histochemical and ultrastructural characteristics of an interstitial cell type different from ICC and resident in the muscle coat of human gut. J Cell Mol Med 2008; 12:1944–1955.
22. Popescu LM, Faussone-Pellegrini MS. Telocytes - a case of serendipity: the winding way from interstitial cells of Cajal (ICC), via interstitial Cajal-like cells (ICLC) to telocytes. J Cell Mol Med 2010; 14:729–740.
23. Horiguchi K, Komuro T. Ultrastructural observations of fibroblastlike cells forming gap junctions in the W/W(nu) mouse small intestine. J Auton Nerv Syst 2000; 80:142–147.
24. Fu M, Tam PK, Sham MH, Lui VC. Embryonic development of the ganglion plexuses and the concentric layer structure of human gut: a topographical study. Anat Embryol 2004; 208:33–41.
25. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res 2005; 319:367–382.
26. Huizinga JD, White EJ. Progenitor cells of interstitial cells of Cajal: on the road to tissue repair. Gastroenterology 2008; 134:1252–1254.